∂c_1), evaluated at $c_1 = 0$)

$$\lim_{c_1 \to 0} s_1 = \frac{M_1(1 - \bar{v}_1\rho)D_{11}}{RT}\left\{1 + c_2\left[\frac{M_2(1 - \bar{v}_2\rho)}{M_1(1 - \bar{v}_1\rho)}\right] \times \left[\frac{\frac{1}{D_{11}}\left(\frac{\partial D_{12}}{\partial c_1}\right)_{c_1,T,P} - \left(\frac{\partial \ln y_1}{\partial c_2}\right)_{c_1,T,P}}{1 + c_2\left(\frac{\partial \ln y_2}{\partial c_2}\right)_{c_1,T,P}}\right]\right\} \quad (7)$$

where y_1 is the activity coefficient of solute 1.

In this form, the result bears a striking resemblance to Svedberg's equation.[8] Measurement of the required quantities presents no unusual difficulty. Provided that $s_1 >> s_2$, the determination of s_1 is straightforward. The Gouy diffusiometer has been used to determine the four diffusion coefficients of several three-component systems.[2,5,6,7] Certain other methods for determining the molecular weight of a solute in a three-component system contain terms of the form $c_2(\partial \ln y_1/\partial c_2)$: for example, light-scattering[12] and sedimentation equilibrium.[13]

I am much indebted to Dr. L. J. Gosting for helpful suggestions and advice.

(12) J. G. Kirkwood and R. J. Goldberg, *J. Chem. Phys.*, **18**, 54 (1950).

(13) M. Wales and J. W. Williams, *J. Polymer Sci.*, **8**, 449 (1952).

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THE STRUCTURAL RELATIONSHIP OF DELTALINE, DELPHELINE AND LYCOCTONINE[1]

Sir:

The alkaloid deltaline has been chemically transformed into delpheline and the latter into desoxylycoctonine, a known degradation product of lycoctonine. These interconversions prove that lycoctonine, delpheline, and deltaline possess the same skeleton structure, and establish the functional relationship of these three important *Delphinium* alkaloids, thus unifying much hitherto unrelated structural evidence. Moreover, we have found that all three alkaloids occur together in *Delphinium barbeyi* Huth, along with traces of several other closely related bases.

Deltaline was isolated as the major base of *D. barbeyi* and *D. occidentale*. It melts at 193.5–194° (evac. cap.; cor.), $[\alpha]^{33}D$ −28.5° (CH_3OH). *Anal.* Calcd. for $C_{27}H_{41}NO_8$: C, 63.88; H, 8.14; N, 2.76; O, 25.22; CH_3 on carbon (3) 8.86; active H (1), 0.20; CH_3O (3), 18.34. Found: C, 63.83; H, 8.21; N, 2.79; O (Unterzaucher), 25.32; CH_3 on carbon (Kuhn–Roth), 7.49; active H (Zerewitinoff), 0.20; CH_3O (Zeisel), 18.75, 18.13. Deltaline was first isolated from *D. occidentale* by Couch.[2] An examination of specimens of deltaline given to one of us (M. C.) by Couch revealed the invariable presence of another alkaloid, delphoccine, not previously reported and not readily separable from deltaline except by chromatography.

(1) Presented before the Section of Pharmaceutical Chemistry and Biochemistry at the Fourth Pan-American Congress of Pharmacy and Biochemistry in Washington, D. C., November 7, 1957.

(2) J. F. Couch, THIS JOURNAL, **58**, 684 (1936).

The presence of delphoccine (whose properties we shall describe elsewhere) in Couch's material accounts for the fact that Couch's formula and constants differ from ours. The functionality of deltaline is $C_{17}H_{18}$(-OCOCH$_3$)(-OCH$_2$O-)(-OCH$_3$)$_3$(>NCH$_2$CH$_3$)(>C-CH$_3$)(OH).[3,4]

Replacement of the hydroxyl group of deltaline with hydrogen and conversion of the acetoxyl group to hydroxyl produces delpheline.[5,6] Treatment of deltaline with highly purified thionyl chloride at room temperature yielded *chloroacetyldelpheline*, m.p. 173.3–173.5° (evac. cap.; cor.), $[\alpha]^{26}D$ −40.7° ($CHCl_3$). *Anal.* Calcd. for $C_{27}H_{40}ClNO_7$: C, 61.64; H, 7.66; Cl, 6.74; N, 2.66; O, 21.29. Found: C, 61.54; H, 7.66; Cl, 6.69; N, 2.65; O (Unterzaucher), 21.09. The reaction of chloroacetyldelpheline with $LiAlH_4$ in refluxing ethyl ether gave an excellent yield of delpheline, identical in m.p., mixed m.p., infrared spectrum, R_f value, and optical rotation with specimens isolated by us from *D. occidentale* and *D. barbeyi* and with a specimen kindly supplied by Dr. R. C. Cookson.

The chromic acid oxidation[7] of lycoctonine [($C_{17}H_{19}$(-OH HO-)(-OCH$_3$)$_4$(>NCH$_2$CH$_3$)(>CCH$_2$OH)] yields the aldehyde, lycoctonal,[7] reducible to the base, desoxylycoctonine, containing two C-methyl groups.

We have synthesized desoxylycoctonine from delpheline in two steps. The secondary hydroxyl group of delpheline, corresponding to the acetoxyl group in deltaline, was methylated by means of sodium hydride and methyl iodide. The resulting *O-methyldelpheline* melts at 102.5–103° (evac. cap.; cor.); $[\alpha]^{24}D$ −6.3° ($CHCl_3$). *Anal.* Calcd. for $C_{26}H_{41}NO_6$: C, 67.37; H, 8.92; N, 3.02; CH_3O (4), 26.78. Found: C, 67.44; H, 9.07; N, 3.19; CH_3O (Zeisel), 24.6. Hydrolysis of the acetal function of O-methyldelpheline with hot 10% sulfuric acid yielded desoxylycoctonine, identical in m.p., mixed m.p., infrared spectrum, and R_f value with the product prepared from lycoctonine by the procedure of Edwards and Marion.[7]

(3) J. Harvey, Jr., Ph.D. Dissertation, University of Pennsylvania, February, 1953; Dissertation Abstr., **13**, 178 (1953); *C. A.*, **48**, 2734[i] (1954).

(4) E. W. Martin, Ph.D. Dissertation, University of Pennsylvania, (1949).

(5) J. A. Goodson, *J. Chem. Soc.*, 665 (1944).

(6) R. C. Cookson and M. E. Trevett, *ibid.*, 984, 2689 (1956).

(7) O. E. Edwards and L. Marion, *Can. J. Chem.*, **30**, 627 (1952).

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RECEIVED DECEMBER 3, 1957

COMPOSITION AND ENZYMATIC SYNTHESIS OF N-ACETYLNEURAMINIC ACID (SIALIC ACID)

Sir:

Previous reports[1,2,3] indicated N-acetylneuraminic acid (NANA) to be an 11 carbon keto acid.

(1) G. Blix, E. Lindberg, L. Odin and I. Werner, *Acta Soc. Med. Upsal.*, **61**, 1 (1956).

(2) E. Klenk, *Angew. Chem.*, **68**, 349 (1956).

(3) A. Gottschalk, *Nature*, **176**, 881 (1955).

Chemical degradation of bovine NANA yielded several products, and one was characterized as N-acetyl-D-glucosamine.[4] NANA from bovine submaxillary mucin was cleaved by extracts of *Vibrio cholerae* to pyruvic acid and an N-acylhexosamine reported to be N-acetylglucosamine.[5] The present studies indicate that human plasma NANA is enzymatically cleaved to pyruvic acid and N-acetyl-D-mannosamine, and that NANA is enzymatically formed by reversal of this reaction.

An enzyme has been purified 110-fold (specific activity = 150 μmoles of pyruvic acid formed/mg. protein/15 min.) from extracts of *Clostridium perfringens* which cleaved the following crystalline sialic acids at the indicated relative rates: NANA (obtained either from human plasma, sheep submaxillary mucin, or *Escherichia coli*),[6] 100; N-glycolylneuraminic acid (pig submaxillary mucin), 65; N,O-diacetylneuraminic acid (bovine submaxillary mucin), 14; methoxyneuraminic acid, 0. Balance studies indicated the stoichiometric formation of pyruvic acid and an N-acylhexosamine from NANA.

Pyruvic acid was determined enzymatically (lactic dehydrogenase) and characterized as the 2,4-dinitrophenylhydrazone (m.p. 216°). The N-acyl-hexosamine (I) was converted to a hexosamine and a steam distillable acid by mineral acid hydrolysis. The organic acid was characterized as acetic acid by conversion to the *p*-bromophenacyl ester (m.p. 83°). The hexosamine was isolated by ion exchange chromatography,[7] and crystallized from methanol as the hydrochloride (II).

Anal. Calcd. for $C_6H_{14}O_5NCl$: C, 33.42; H, 6.54; N, 6.50; Cl, 16.44. Found; C, 33.52; H, 6.60; N, 6.24; Cl, 16.35.

Compound II was characterized as D-mannosamine, HCl in the following manner: (1) The acetylacetone reagent yielded a color with II which is typical for hexosamines. The relative molar absorbancies obtained with D-glucosamine, HCl; synthetic D-mannosamine, HCl[8]; and II was 1.00, 0.81, and 0.84, respectively. (2) Treatment of II with ninhydrin at *p*H 5.0 (citrate buffer)[9] yielded a pentose characterized as D-arabinose by paper chromatography and conversion to D-arabo-benzimidazole (m.p. 235°; $[\alpha]^{25}D$ −40° which corresponded to authentic derivative prepared in the same manner). (3) II exhibited $[\alpha]^{25}D$ −1.5° (*c* 0.8% in water); synthetic mannosamine, HCl exhibited the same value under similar conditions. (4) The X-ray powder diffraction pattern obtained with II differed markedly from that obtained with D-glucosamine, HCl but was identical with that obtained with D-mannosamine, HCl.

A general procedure for quantitative N-acetylation of hexosamines[10] was applied to II yielding an N-acetylhexosamine (III), and to D-mannosamine, HCl yielding N-acetyl-D-mannosamine; neither III nor N-acetyl-D-mannosamine has yet been crystallized. Compounds I, III and N-acetyl-D-mannosamine yielded approximately 55% of the molar absorbancy obtained with N-acetyl-D-glucosamine in the modified Morgan–Elson reaction.[11] In several solvent systems, I and III were not separable from N-acetyl-D-glucosamine, but on borate-treated paper[12] they migrated at 0.38 the rate obtained with N-acetyl-D-glucosamine. With ketose reagents, both II and III yielded less than 3% of the molar absorbancy obtained with D-fructose.

The enzymatic synthesis of NANA is indicated in Table I.

TABLE I

ENZYMATIC SYNTHESIS OF N-ACETYLNEURAMINIC ACID

Pyruvic acid[a]	Incubation time, min.	NANA formed,[b] μmoles/ml.
N-Acetylglucosamine or N-acetylgalactosamine	0	0.00
	60	0.00
Compounds I or III	0	0.00
	60	1.25
Synthetic N-acetylmannosamine	0	0.00
	60	1.29

[a] The reaction mixtures contained/ml.: 20 μmoles of N-acetylhexosamine; 25 μmoles of potassium pyruvate; 120 μmoles of potassium phosphate buffer, *p*H 7.1; 25 γ of protein. Omission of protein yielded no NANA. [b] Determined by direct Ehrlich reaction and with the Bial reagent[13]; the product corresponded to NANA by paper chromatography (*n*-butanol, acetic acid, water: 4, 1, 5).[1] The colorimetric methods are sensitive to 0.02 μmole NANA/ml.

NANA was formed when the purified enzyme was incubated with pyruvic acid and either I, III, or synthetic N-acetyl-D-mannosamine, but not with N-acetyl-D-glucosamine or N-acetyl-D-galactosamine. Similar results were obtained with the N-acylhexosamine isolated after enzymatic cleavage of the bacterial NANA. At equilibrium, the molar ratio of NANA to either of the cleavage products was 1:9. The enzymatic and chemical data characterize I as N-acetyl-D-mannosamine. In view of the specificity of the enzyme, it appears likely that sheep submaxillary mucin and *E. coli* NANA are identical with human plasma NANA. The decreased rates of cleavage of pig and bovine sialic acids may be due to differences in the structure of the 9 carbon chain (*e.g.*, glucosamine rather

(4) R. Kuhn and R. Brossmer, *Chem. Ber.*, **89**, 158 (1956).

(5) R. Heimer and K. Meyer, *Proc. Nat. Acad. Sci. U. S.*, **42**, 728 (1956).

(6) A culture of *E. coli* K-235, which produces colominic acid, was kindly supplied by Dr. W. F. Goebel; G. T. Barry and W. F. Goebel, *Nature*, **179**, 206 (1957). NANA recently has been obtained from colominic acid; G. T. Barry, presented before a meeting of the U. S. Nat. Acad. of Sciences, New York, November, 1957, Abstracts of Papers, p. 4. In this laboratory, NANA was isolated after mild acid hydrolysis of the bacterial cells.

(7) S. Gardell, *Acta Chem. Scand.*, **7**, 207 (1953); this method is commonly employed for the separation of D-glucosamine and D-galactosamine. Under the standard conditions, D-mannosamine does not separate completely from D-glucosamine (the peaks intersect). The acid hydrolysate of I yielded a single peak which coincided exactly with the peak obtained with authentic D-mannosamine. Acid hydrolysis of 0.37 mmole of I (derived by enzymatic cleavage of 0.40 mmole of NANA), followed by chromatography, yielded 0.33 mmole of hexosamine in the peak. The first crop of crystalline II weighed 40 mg.

(8) P. A. Levene, *J. Biol. Chem.*, **39**, 69 (1919).

(9) H. G. Pontis, *ibid.*, **216**, 195 (1955); P. J. Stoffyn and R. W. Jeanloz, *Arch. Biochem.*, **52**, 373 (1954).

(10) S. Roseman and J. Ludowieg, THIS JOURNAL, **76**, 301 (1954); S. Roseman and I. Daffner, *Anal. Chem.*, **28**, 1743 (1956).

(11) J. L. Reissig, J. L. Strominger and L. F. Leloir, *J. Biol. Chem.*, **217**, 959 (1955).

(12) C. E. Cardini and L. F. Leloir, *ibid.*, **225**, 317 (1957).

(13) I. Werner and L. Odin, *Acta Soc. Med. Upsal.*, **57**, 230 (1952).

than mannosamine) but more likely due to the known differences in the acyl groups.

While D-mannosamine has been synthesized previously, this apparently is the first report of its natural occurrence. Nevertheless, the compound may be widespread in nature; most of the techniques employed for characterization of D-glucosamine would not distinguish it from D-mannosamine.[14]

(14) We are deeply grateful to Drs. Edwin A. Popenoe and Ruth Drew who informed us that extracts of *Cl. perfringens* exhibited the desired enzymatic activity with NANA and who supplied us with a culture of the organism. Dr. G. Blix kindly supplied the sialic acid samples from bovine, pig and sheep submaxillary mucins; the methoxyneuraminic acid was a gift of Dr. E. Klenk.

(15) Postdoctoral fellow, American Cancer Society.

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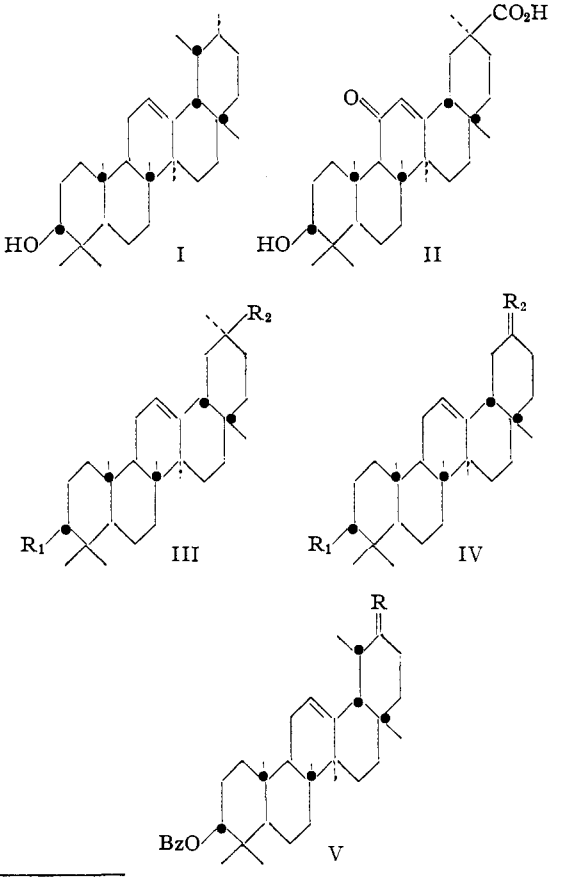
PROOF OF THE STRUCTURE AND STEREOCHEMISTRY OF α-AMYRIN BY SYNTHESIS FROM A β-AMYRIN DERIVATIVE, GLYCYRRHETIC ACID

Sir:

The gross structure of α-amyrin which had been determined by Ruzicka, Jeger and co-workers[1] (I without stereochemical connotations) was elaborated to the complete stereochemical description I in 1954 on the basis of extensive chemical and physical data.[2] Subsequently a number of other formulations were advanced.[3-5]

We now report the unambiguous confirmation of expression I by synthesis of α-amyrin from glycyrrhetic acid,[6] a known derivative of β-amyrin having structure II.

Hydrogenation of methyl glycyrrhetate, m.p. 242.5–249.5°, $[\alpha]_D$ +153, afforded the 11-desoxy derivative, m.p. 233–245°, $[\alpha]_D$ +115°, which was saponified and acetylated to 11-desoxyacetylglycyrrhetic acid, m.p. 305–307°, $[\alpha]_D$ +117°. This was converted to the isocyanate (III, R_1 = OAc, R_2 = N=C=O), strong infrared max. 2265 cm.$^{-1}$, via the acid chloride and the azide.[7] Reduction of the isocyanate ($LiAlH_4$) yielded the amine III, R_1 = OH, R_2 = $NHCH_3$, m.p. 216–229.5°, $[\alpha]_D$ +99°; found: C, 81.29; H, 11.90; N, 3.40, which was methylated to the quaternary iodide III, R_1 = OH, R_2 = $^{\oplus}N(CH_3)_3$. The olefin IV, R_1 = OH, R_2 = CH_2, m.p. 166–169°, $[\alpha]_D$ +157°; found: C, 84.28; H, 11.21; infrared max. 890 cm.$^{-1}$ (strong), 1652 cm.$^{-1}$, 3620 cm.$^{-1}$, formed from the quaternary salt with potassium *t*-butoxide, was acetylated (acetate, m.p. 196.5–197.5°, $[\alpha]_D$ + 164°; found: C, 82.42; H, 10.80), and degraded to the ketone IV, R_1 = OAc, R_2 = O, by hydroxylation of the terminal double bond with osmium tetroxide and cleavage with periodic acid (found for IV, R_1 = OAc, R_2 = O: m.p. 242.5–244°, $[\alpha]_D$ +85.5°; C, 79.22; H, 10.22, infrared max., 1724, 1740 cm.$^{-1}$ (both strong)). The acetoxy ketone was saponified and benzoylated to give IV, R_1 = OBz, R_2 = O, m.p. 229.5–231.5°, $[\alpha]_D$ +100°, found: C, 81.48; H, 9.70, which was monomethylated using trityl sodium and methyl iodide to V, R = O, m.p. 248–250.5°, $[\alpha]_D$ +115°; found: C, 81.56; H, 9.65.[8] The attachment of the added methyl to C_{19} and not C_{21}, anticipated from analogy to the coprostanone series, was established by rotatory dispersion measurements.[9] Reaction of V, R = O, with methylene-triphenylphosphine[10,11] led to the terminal olefin V, R = CH_2, m.p. 224–226°, infrared max. 1718, 1644, 888



(1) A. Meisels, O. Jeger and L. Ruzicka, *Helv. Chim. Acta.*, **32**, 1075 (1949); see also O. Jeger, *Fortschritte der Chemie Organische Naturstoffe*, **7**, 1 (1950).

(2) E. J. Corey and J. J. Ursprung, *Chem. and Ind.*, 1387 (1954), THIS JOURNAL, **78**, 183 (1956). Previously the configurations at C_9, C_{14}, C_{17}, C_{18}, C_{19} and C_{20} were unknown, although the configuration at C_{17} opposite to that in I had been considered as proved on the basis of lengthy degradative sequences [O. Jeger, *Angew. Chem.*, 196 (1951); see also *Ann. Rep.*, **48**, 198 (1951)].

(3) J. L. Beton and T. G. Halsall, *Chem. and Ind.*, 1560 (1954).

(4) F. S. Spring and co-workers, *ibid.*, 281 (1955); *J. Chem. Soc.*, 2606, 2610, 3072, 3371, 3378, 3992 (1955); *ibid.*, 456, 465 (1956). These papers also describe correlation of α- and β-amyrin at C_{14} and C_{17}. See also D. D. Phillips and D. E. Tuites, THIS JOURNAL, **78**, 5438 (1956).

(5) G. D. Meakins, *Chem. and Ind.*, 1353 (1955).

(6) L. Ruzicka and co-workers, *Helv. Chim. Acta*, **19**, 1402 (1936); **20**, 312, 1271 (1937); **22**, 195 (1939); J. M. Beaton and F. S. Spring, *J. Chem. Soc.*, 3126 (1955).

(7) See H. H. Zeiss and W. B. Martin, THIS JOURNAL, **75**, 5935 (1953).

(8) This technique is recommended for the monomethylation of ketones in high yield. The excess trityl sodium rapidly and completely converts ketone to mono-enolate and is rapidly destroyed by the excess methyl iodide.

(9) *Vis.* by comparison of IV, R_1 = OBz, R_2 = O and V, R = O with coprostanone and 2- and 4-methylcoprostanones, kindly made by Dr. Carl Djerassi.

(10) G. Wittig and U. Schöllkopf, *Ber.*, **87**, 1318 (1954).

(11) F. Sondheimer and R. Mechoulam, Abstracts, A.C.S. Meeting, April 1957, p. 35-O.